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July 21, 2011

Kenneth Williams
LUNA US LLC
5795 S. Sandhill Road, Suite F
Las Vegas, NV 89120

Dear Mr.Williams:

RTI is pleased to submit this letter report to LUNA US LLC on our project to test the efficacy of microbial inactivation by the LUNA LNT2 6000 Photocatalytic Oxidation Air Purification System. This air cleaner unit was supplied by LUNA US LLC and tested as supplied with the unit operated at two settings, medium and high. The testing program included four organisms: two bacteria, and two fungi. *Staphylococcus epidermidis* is a common gram-positive human-shedding organism and was the representative vegetative bacterium. *Mycobacterium parafortuitum* was the other bacterium which belongs to the same genus as and has physical characteristics similar to *Mycobacterium tuberculosis,* the causative agent of tuberculosis. Because *Mycobacterium tuberculosis* is primarily spread from person to person in respiratory droplets, the *M. parafortuitum* bioaerosol was generated in artificial sputum to most closely simulate the coating that *Mycobacterium tuberculosis* would have in the environment when expelled by coughing. The other three organisms were generated in water. *Aspergillus versicolor* and *Penicillium chrysogenum,* the representative fungi, are frequently reported as causative agents of hypersensitivity pneumonitis and have been isolated from a number of problem buildings.

## TEST METHOD

### Chamber Air Cleaner Test

The test for each organism included a natural decay measurement and an air cleaner decay measurement of the air cleaner operating at the medium and also at the high setting. All three measurements were performed after filling the chamber with challenge bioaerosol. The natural decay is defined as the decay of the test bioaerosol in the chamber with the air cleaner off. The air cleaner decay measurement is defined as the decay while the air cleaner is running. For the testing, the air cleaner was positioned horizontally in the center of the chamber at a height of 81 cm above the floor.

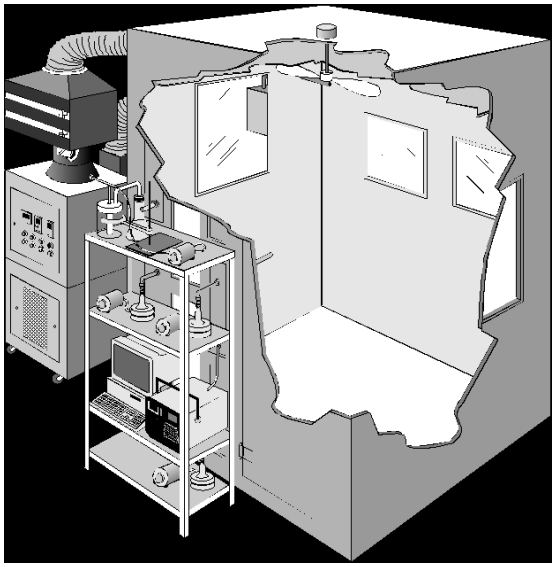
The test method has been described in depth by Foarde et al. (1999). As an overview, the paper describes a test method to determine a Clean Air Delivery Rate (CADR) type measurement for a device when challenged with microbiological aerosols. The method is a modification of the Association of Home Appliance Manufacturers (AHAM) Standard AC-1, “Standard Method for Measuring Performance of Portable Household Electric Cord-Connected Room Aircleaner” which determines the CADR for three different particulate matter challenges (smoke, dust, and

pollen). This extension of the AHAM method to microbial aerosols follows the tradition of the AHAM test of using realistic particle challenges and provides a means to compare and evaluate different brands of room air cleaning devices regarding characteristics significant to product use. This is a useful approach for evaluating a wide range of devices.

**Test Chamber and Bioaerosol Sampling:**
The Dynamic Microbiological Test Chamber (DMTC) was used for the air cleaner tests. The DMTC is a room-sized environmental chamber contained within the microbiological aerosol test facility, a nominally Class 1,000 cleanroom. The chamber is 2.44 x 2.44 x 3.05 m (18.16 $m^3$ or 640 $ft^3$). The walls and containment ceiling are 10 cm thick prefabricated panels with a stainless steel interior layer. The floor of the chamber was custom constructed of 12-gauge stainless steel with welded seams and insulation underneath between the support members. Floor seams were polished and the coved corners were sealed. The ceiling-mounted mixing fan consists of a two-blade aluminum casting 61 cm in diameter attached to a shaft extending 61 cm from the ceiling into the center of the chamber. To reduce the difficulty of decontaminating the interior features, no electrical outlets were installed inside the chamber. A finished 5 cm penetration in one wall allows extension cord access through rubber stoppers.

Temperature and humidity control were provided by a separate external air handler (AHU). The AHU also controls the steam humidifier which adds water to the chamber air while the HVAC system removes some water and controls the air temperature. Airflow through the system is monitored by an airflow station and controlled by a blower speed controller with the AHU. Air cleaning of the chamber is attained through the use of a HEPA (High Efficiency Particulate Air) filter installed on the discharge side of the AHU. It contains both an ASHRAE 30% prefilter and a HEPA filter.

Figure 1 shows an artist's rendition of the DMTC configured for air cleaner testing. The Model LUNA LNT2 6000 air cleaner was positioned in the center of the chamber.



The challenge *M. parafortuitum* bioaerosol suspensions were aerosolized using a Spraying Systems Co. two-fluid spray nozzle. The bacterial sample supplied to the spray nozzle was suspended in artificial sputum designed to mimic the conditions present in droplets expelled from the human respiratory system. The spray nozzle generates droplets directly into the top of a cylindrical pre-chamber referred to as a drying tower. The drying tower, supplied with dry, HEPA-filtered air, was used as a mixing and drying space for the test aerosol. After leaving the drying tower, the bioaerosol passed through a TSI Model 3012A aerosol neutralizer (Krypton-85 radioactive source) and into the inlet to the DMTC which was positioned near the middle of a wall that is adjacent to the dynamic chamber sampling wall. The Model 3012A neutralized the electrostatic charge on the aerosol, an unavoidable consequence of most aerosol-generation methods, producing an aerosol with a normalized charge distribution similar to that found in room or ambient air.

The challenge *S. epidermidis, A. versicolor* and *P. chrysogenum* bioaerosol suspensions were aerosolized using a Collison modified MRE-type six-jet nebulizer (BGI, Waltham, MA). The Collison nebulizer generated particles or droplets with an approximate mean diameter of 2 μm. These droplets were injected directly into the side of the drying tower, and also passed through the aerosol neutralizer.

Extractive sampling of the bioaerosols was accomplished using ports placed in sampling panels located in one wall of the chamber (see Figure 1). Three sampling ports were used to collect triplicate simultaneous samples. Port A was positioned near the center of the chamber wall, 1.52 m above the floor of the chamber and 1.0 m from the front wall. Port B was 1.52 m above the floor but was 0.25 m from the front wall of the chamber. The third port, C, was directly below Port A, but 0.65 m above the floor of the chamber. Stainless steel 1.27 cm diameter piping extending 0.76 m into the middle of the chamber was used as sample lines. The dimensions of the sample lines were chosen to minimize particle losses during sampling.

Sampling of the *M. parafortuitum* was accomplished using all-glass impingers (AGI-4). After sampling, the impinger fluid was diluted (if necessary) and plated for viable *M. parafortuitum* bacteria present. Plates were incubated for several days at 37°C. CFUs (colony forming units) were then enumerated and their identity confirmed.

Sampling of *S. epidermidis, A. versicolor* and *P. chrysogenum* was accomplished using one-stage Andersen viable bioaerosol samplers loaded with Petri dishes containing growth media. The one-stage Andersen sampler is a 400-hole multiple-jet impactor operating at 28 L/min. A positive hole correction was used to adjust colony counts from the Andersen multiple-hole impactor for the possibility of collecting multiple colonies through a hole[1]. After sampling, the Petri dishes were removed from the sampler and incubated overnight at 37°C for *S. epidermidis*, and several days at 23°C for *A. versicolor* and *P. chrysogenum* CFUs (colony forming units) were then enumerated and their identity confirmed.

**Test Protocol:**

The test protocol for *M. parafortuitum* was as follows:

1) Turn on the chamber AHU and circulating fan.
2) Allow the HEPA to clean the chamber air for at least 1 hour.
3) Turn off AHU and turn on air and liquid flow to the aerosol generation system and aerosolize the *M. parafortuitum* in artificial sputum suspension into the chamber with HEPA-filtered drying air.
4) Run sterile distilled water through the aerosol generation system into the drying tower together with drying air. Then turn off the aerosol generation system and drying air, and close the valve between the drying tower and the chamber to prevent backflow. .
5) Next turn off the circulating fan in the chamber one minute prior to the start of collection for the "0 min" sample.
6) Switch on the air cleaner at the start of collection for the "0 min" sample. Verify that the lights are visible on the units display which confirm operation at the appropriate setting (one set of lamps for medium, and two for high).
7) Collect triplicate bioaerosol measurements at appropriate intervals (0, 5, 10 and 15 minutes).

In the natural decay test for *M. parafortuitum*, step 6 was omitted.

The test protocol for *S. epidermidis, A. versicolor* and *P. chrysogenum* was as follows:
1) Turn on the chamber AHU and circulating fan.
2) Allow the HEPA to clean the chamber air for at least 1 hour.
3) Turn off AHU and turn on the Collison nebulizer and aerosolize the organism suspension using HEPA-filtered drying air. Turn off Collison nebulizer, and continue to allow the drying air to flow through the drying tower and into the chamber.
4) One minute prior to the start of collection for the "0 min" sample turn off the drying air, close the valve between the drying tower and the chamber to prevent backflow, and turn off the circulating fan in the chamber.
5) Switch on the air cleaner at the start of collection for the "0 min" sample. Verify that the lights are visible on the units display which confirm operation at the appropriate setting (one set of lamps for medium, and two for high).
6) Collect triplicate bioaerosol measurements at appropriate intervals (usually 0, 5, 10 and 15 minutes or 0, 20, 40 and 60 minutes).

In the natural decay test for *S. epidermidis A. versicolor* and *P. chrysogenum*: step 5 was omitted.

**Calculations:**

The performance of the air cleaner was evaluated by determining the Clean Air Rate (Microbial) or CARm, calculated as the CADR in the AHAM method. To calculate the CARm, the measured decay ($k_e$) and natural decay ($k_n$) rates are first calculated using the formula:

$$k = \frac{(\Sigma\, t * \ln C_t) - [(\Sigma\, t)(\Sigma \ln C_t)]/n}{(\Sigma\, t^2) - (\Sigma\, t)^2 / n} \qquad \text{Equation 1}$$

where:

$C_t$ = concentration at time, t
n = number of data points used in the regression
k = decay constant ($time^{-1}$)
t = time (minutes)

Then the CAR(m) was calculated for each measured decay rate, using the formula:

$$CARm = V k_e - k_n \qquad \text{Equation 2}$$

where:
V = volume of the test chamber ($ft^3$)
$k_e$ = measured decay rate ($min^{-1}$)
$k_n$ = average natural decay rate ($min^{-1}$) for an organism.

RESULTS

Chamber Air Cleaner Test

The Model LUNA LNT2 6000 air cleaner was tested as provided by LUNA US LLC. For each device on test, the unit was run at the medium and the high setting and verification was made that the appropriate lights were visible on the unit's display as confirmation.

Figures 2, 3, 4 and 5 show the decay curves for *S. epidermidis, M. parafortuitum*, *A. versicolor* and *P. chrysogenum,* respectively, for the Model LUNA LNT2 6000 air cleaner. The numbers of CFUs per cubic foot in the chamber are plotted on the y-axis with the time in minutes on the x-axis. The data points for each time represent the average results from the three sampling locations. The error bars indicate the standard deviations calculated for the multiple samples comprising each average. Note that for many of the data points, the standard deviations are sufficiently tight that they do not appear separate from the data points. The natural decay curves are labeled "Device OFF", while the air cleaner decay curves (with the air cleaner running at the medium setting) are labeled "Device MED" and the air cleaner decay curves (with the air cleaner running at the high setting) are labeled "Device HIGH"

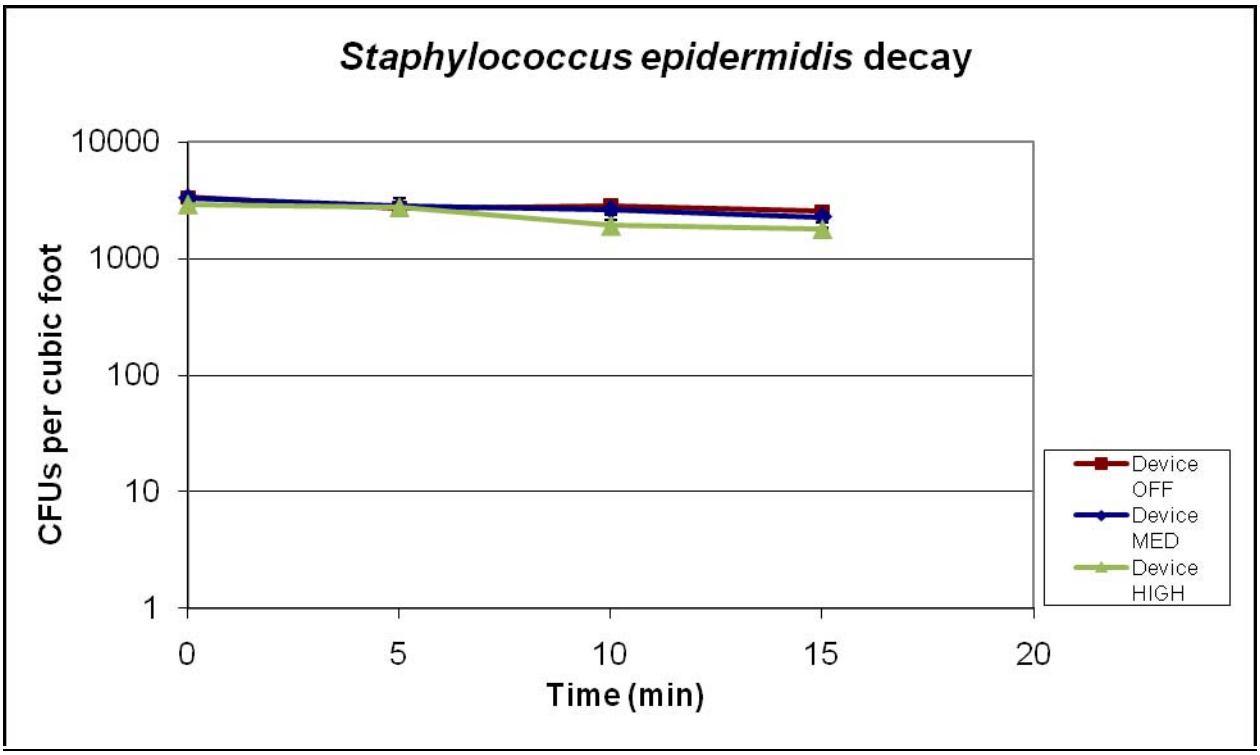



**Figure 2. Decay curves for *S. epidermidis***

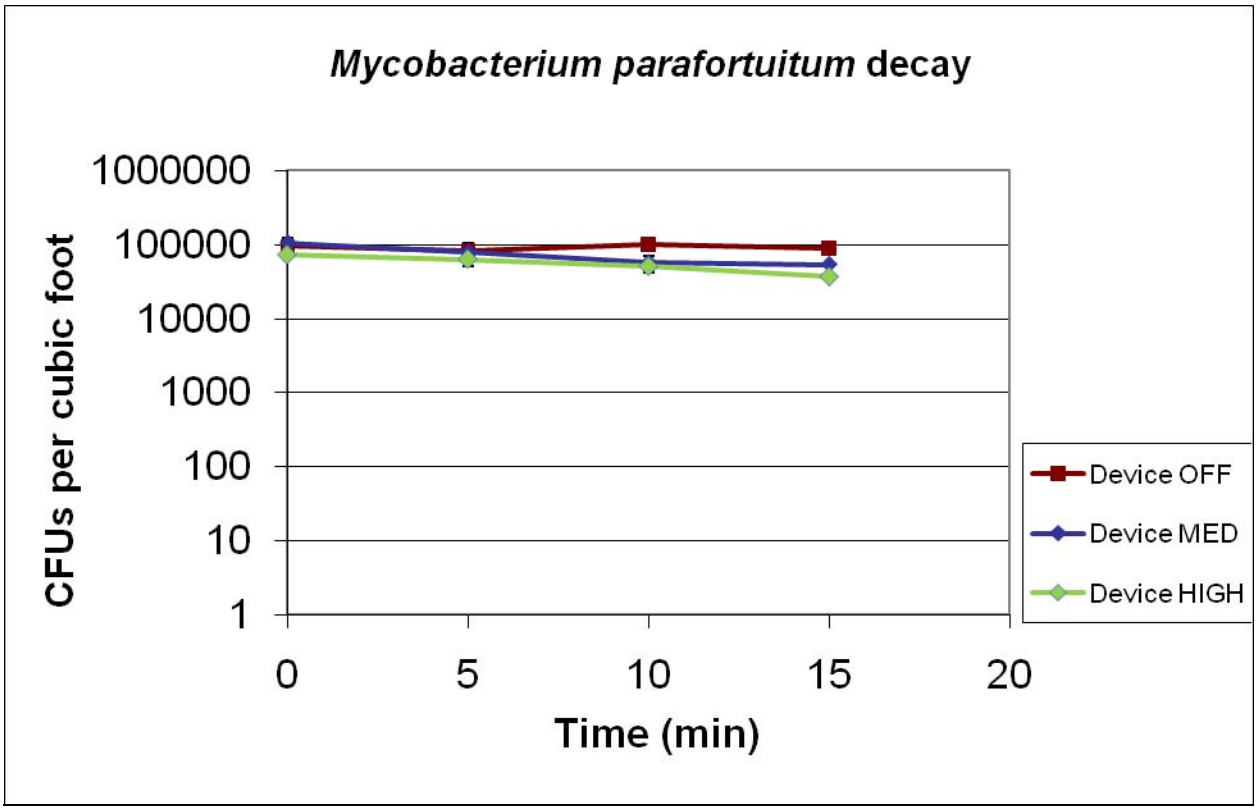



**Figure 3. Decay curves for *M. parafortuitum***

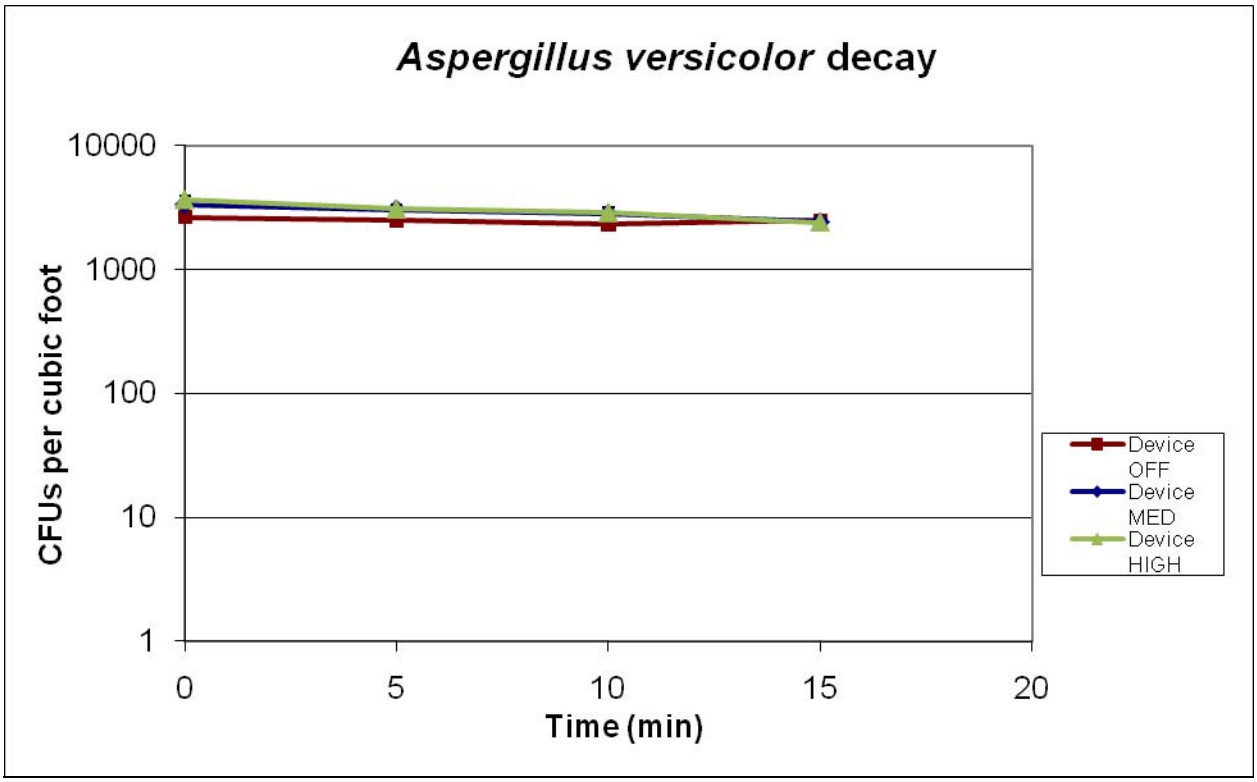



**Figure 4. Decay curves for *A. versicolor***

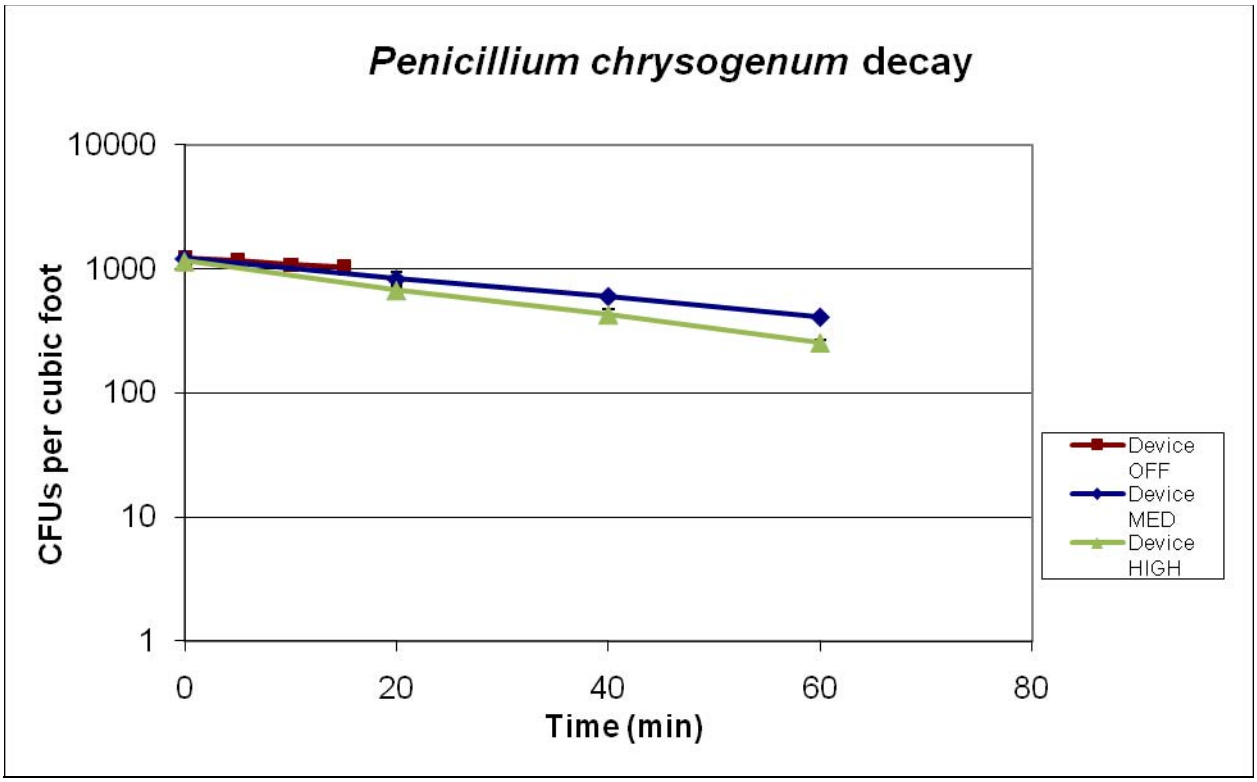



**Figure 5. Decay curves for *P. chrysogenum***

In each case, the impact of the LUNA LNT2 6000 air cleaner is visible in the graph. The decay rates with the device on are higher than the decay rates with the device off over the time periods observed. The actual natural decay ($k_n$) and measured decay ($k_{MED}$) and ($k_{HIGH}$) rates for each organism at each air cleaner setting calculated according to Equation 1 are shown in Table 1 and 2. Table 1 provides the data for the Medium setting and also presents the CARm results and standard deviations for each organism. Table 2 provides the data for the High setting and also presents the CARm results and standard deviations for each organism. The CARm was calculated as shown in Eq. 2 and is a comparison of the two decay rates (natural and air cleaner) as a function of the volume of the test chamber (640 $ft^3$). These results are also displayed graphically in Figures 6 and 7.

**Table 1. Decay rates and CARm values for introduced bioaerosols at the Med setting**

| | *S. epidermidis* | *M. parafortuitum* | *A. versicolor* | *P. chyrsogenum* |
|---|---|---|---|---|
| kn | -0.016 | 0.000 | -0.005 | -0.012 |
| $k_{MED}$ | -0.024 | -0.046 | -0.021 | -0.018 |
| CARm | 5 | 30 | 10 | 4 |
| s.d. | 0.22 | 0.49 | 0.15 | 0.14 |

**Table 2. Decay rates and CARm values for introduced bioaerosols at the High setting**

| | *S. epidermidis* | *M. parafortuitum* | *A. versicolor* | *P. chyrsogenum* |
|---|---|---|---|---|
| kn | -0.016 | 0.000 | -0.005 | -0.012 |
| $K_{HIGH}$ | -0.036 | -0.044 | -0.027 | -0.025 |
| CARm | 13 | 28 | 14 | 8 |
| s.d. | 0.23 | 0.43 | 0.16 | 0.14 |

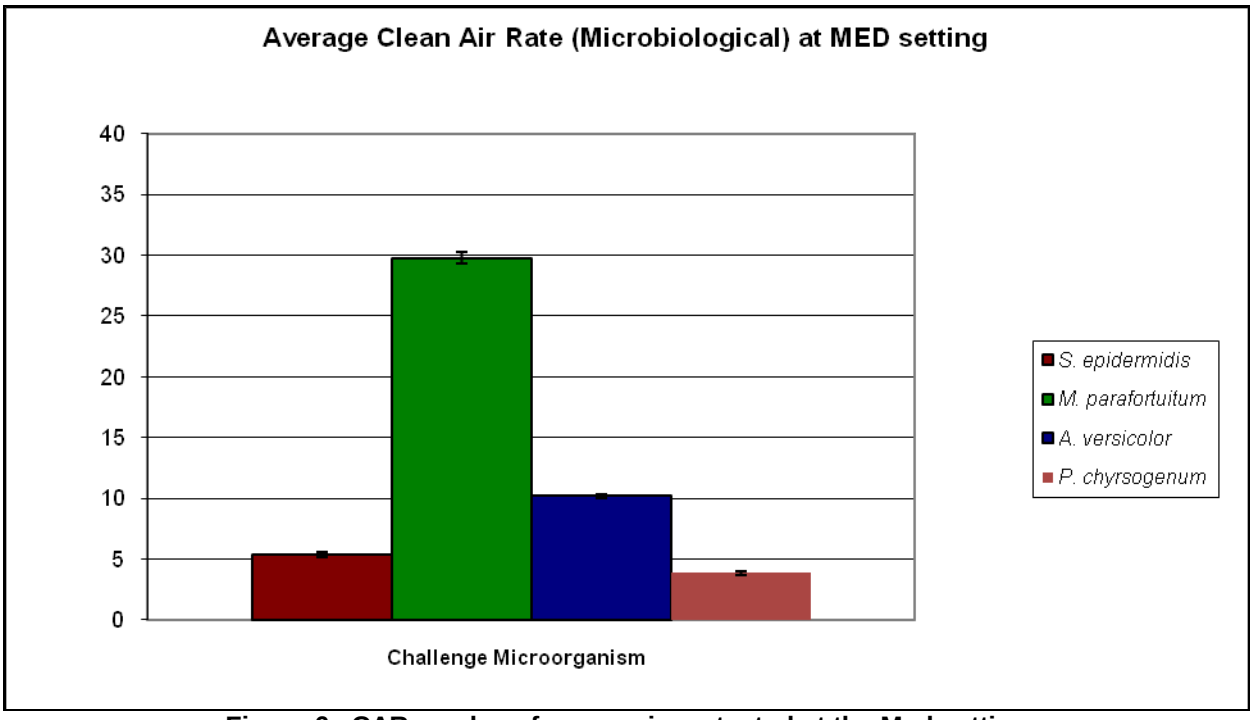



**Figure 6. CARm values for organisms tested at the Med setting**

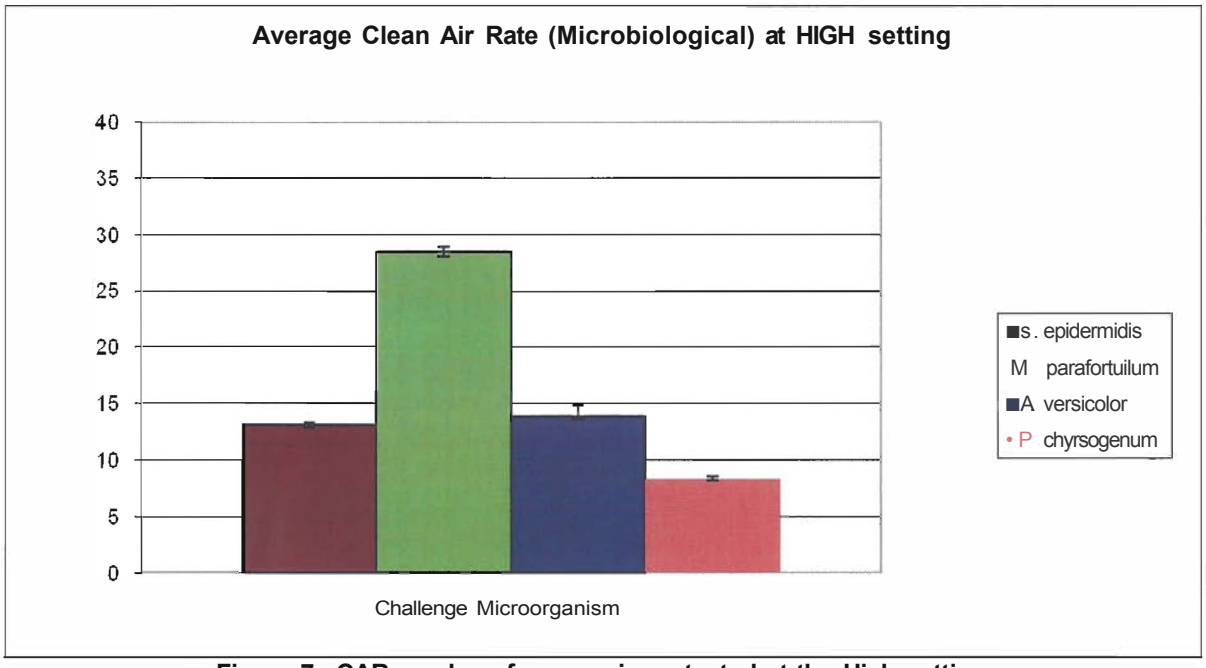



**Figure 7. CARm values for organisms tested at the High setting**

In the ideal case where the air cleaner provides a well-mixed chamber, the CARm is equivalent to the product of the air cleaner's flow rate and its inactivation/filtration efficiency for the challenge bioaerosol. In a chamber test, however, the test chamber is only "well-mixed" to the extent that the device itself provides this mixing by the air motions generated by its fan. Thus, the CARm combines the effects of the efficiency of the air cleaner and the effectiveness of the air cleaner to draw the test chamber's air through it. Generally, the CARm should not exceed the air cleaner flow rate. For the Model LUNA LNT2 6000 air cleaner, the results show that the CARm values for the bioaerosol challenges tested ranged from 13% to 100% of the air flow rate which was given in the product manual as 30 CFM.

Please let me know if you have any additional questions, and feel free to call me at 919-541-7349 or email me at rnherman@rti.org.

Sincerely,

*v:_u/l..*

Michael L. Herman
Microbiologist 3
Center for Microbial Community Systems and Health Research

REFERENCES

1. Macher, J.M. 1989. Positive Hole Correction of Multiple-jet Impactors for Collecting Viable Microorganisms, *American Industrial Hygiene Association Journal.* 50: 561-568.